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ELI LILLY AND COMPANY

By KS Rhoades Date 5-18-04

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Jeffrey Clayton Baker, *et al.*	
Serial No.	:	09/744,432	
Filed	:	January 22, 2001	Group Art Unit: 1653
For	:	Cryogranulation of Activated Protein C	Examiner: A. Mohamed
Docket No.	:	X-11634	

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jeffrey Clayton Baker, Ph.D., declare that:

1. I received a B.A. in Biochemistry and Molecular Biology from Northwestern University, Evanston, Illinois, in 1980, and a Ph.D. in Biochemistry from North Texas State University, Denton, Texas, in 1984. My postdoctoral work was conducted in the laboratory of Dr. Bruce N. Ames at the University of California, Berkeley, California, in the Department of Biochemistry from 1984 through 1988.

In 1988, I began working for Eli Lilly and Company, Indianapolis, Indiana, as a Senior Biochemist. I was promoted to Research Scientist in 1993 and advanced to Senior Research Scientist in 1997. In 2001, I was selected as one of eight senior contributors to a global technical policy and review group reporting through the Vice President for Quality at Eli Lilly and Company and given the title of Quality Advisor – Validation, Global Quality Policy, and Technical Support. This group was dissolved in 2002 as a result of corporate reorganization. From 2002 through early 2004, I held the title of Senior Research Scientist –

Validation, Manufacturing Science, and Technology, with responsibilities for the technical development and assessment of validation and qualification standards for global manufacturing. In April 2004, my title was changed to Research Advisor. My work has focused on protein process research and development at laboratory, pilot plant, and production levels as well as protein manufacturing control and validation strategies.

I have been involved in biochemistry research and development for more than twenty-four years and in protein process research and development for more than fifteen years, authoring or co-authoring over ten publications and three posters on protein topics. I am a co-inventor on 9 patents, have been invited to present over twenty seminars, contributed to numerous global regulatory commitment documents or presentations on the development of biosynthetic processes to U.S. and other regulatory authorities, have taught biochemistry-related topics at universities as well as in industry, and belong to several professional associations. I received various honors over my career, including two Predoctoral Fellowships and two Lilly Research Laboratories President's Recognition Awards (see attached curriculum vitae).

2. I am an inventor named in this application.

3. I have reviewed U.S. Patent No. 5,716,645 (the '645 patent) and U.S. Patent No. 5,516,650 (the '650 patent). The '650 patent contains a reference to lyophilizing the protein C or activated protein C of that patent's invention. The '645 patent includes use of cryoprecipitated plasma to prepare a fibrinogen composition essentially free of Factor VIII. The '645 patent also uses lyophilization for fibrinogen.

4. Cryogranulation and lyophilization are two distinct processing techniques, differing in a variety ways. For example, the end product produced using cryogranulation is a frozen liquid containing both the desired product and water in the form of discrete granules from solutions or slurries of bulk drug substances after contact with a cryogenic material such as liquid nitrogen (Schmidt *et al.*, *BioPharm*, 28-32 (1997)). Alternatively, lyophilization extracts the water from the product, leaving a readily handled solid product plug. Lyophilization is also referred to as freeze drying since it involves the process of isolating a solid substance from solution by freezing the solution and evaporating the ice under vacuum (see http://cancerweb.ncl.ac.uk/cgi-bin/omd?query=lyophilization&action=Search+OMD).

Additionally, the physical nature of the product differs given the technique used. Cryogranules are frozen pellets that can roll, spill, or break. On the other hand, lyophilized products are solid powders that can flow, cake, or be milled. Various temperatures affect cryogranulated products. At high temperatures, cryogranulated products boil. Cryogranules transform into a liquid at room temperature, but remain frozen solids at the proper cold temperature. Alternatively, lyophilized products are solid at any temperature.

Also, chemical stability is linked to different factors for cryogranulated products and lyophilized products. Cryogranulated products have uniform hydration, with their chemical stability linked to temperature. While most of the water is removed from lyophilized products, trace amounts of water can remain. The degree of hydration can vary between products and between production lots of the same product. Thus, chemical stability is linked to hydration for lyophilized products.

Furthermore, the process for preparing cryogranules and lyophilized products are quite different. Cryogranules are generated by contacting droplets of the solution containing the product in liquid nitrogen or other freezing agents suitable for rapidly freezing the solution at temperatures from –40°C to –90°C (Schmidt *et al.*, (1997)). Discrete frozen pellets are produced during the residence time in which the solution is in contact with liquid nitrogen (*Id.*). As such, cryogranulation is a partitioning technique, where the product is merely divided into small frozen units. Alternatively, lyophilization is the process of isolating a solid substance from solution by freezing the solution and sublimating the ice under vacuum, as noted above (see http://cancerweb.ncl.ac.uk/cgi-bin/omd?query=lyophilization&action=Search+OMD). Thus, lyophilization is a separation technique, where the product is separated as a solid from the liquid.

5. The '645 patent is directed to cryoprecipitation, a purification technique used to facilitate the separation of a solid from a liquid, not cryogranulation. Even if the '645 patent included teachings on cryogranulation, cryogranulation of other protein products does not suggest the viability of using cryogranulation as a processing technique for activated protein C. For any freezing methodology, various issues must be addressed, including the effect of freezing rate on product stability (Ryan *et al.*, *BioPharm*, 32-38 (1995)). In that reference, Ryan *et al.* reported on the physical attributes of cryogranulated cell pastes and inclusion body pastes derived from fermentation of *E. coli* producing recombinant human proteins, not their product stability. However, Schmidt *et al.* reported that a wide variety of cryogranulated molecules, including a tripeptide, a small protein

(approximately 6,000 MW), a large protein (>60,000 MW), an enzyme intermediate, and vancomycin hydrochloride, showed excellent stability (Schmidt *et al.*, (1997)). Such a conclusion was available only after performing the experiments on each of the molecules. Schmidt *et al.* notes that "[a]s the era of biotechnology continues to advance, new technologies such as cryogranulation must be developed and implemented to facilitate the processing of reactive biomolecules from the bulk stage to the finished product dosage form while maintaining stability, purity, and other quality attributes." (*Id.*). In the absence of experimentation, the activity and stability of a protein subjected to any freezing technique, including cryogranulation conditions, cannot be predicted. With any protein, but particularly proteins used for pharmaceutical products, experimentation is necessary to ascertain whether or not a particular protein's activity is retained and recovered at a level sufficient to maintain commercial utility. As such, the flash freezing technique employed in cryogranulation can have unique effects on different proteins and must be tested on a given protein in order to determine whether or not cryogranulation is a feasible processing technique. Therefore, successfully using cryogranulation for activated protein C is not predictable from use of this technique for other protein products.

6. The '645 patent includes use of cryoprecipitated plasma to prepare a fibrinogen composition essentially free of Factor VIII. Activated protein C is quite different from plasma, fibrinogen, and Factor VIII. In fact, the percent sequence identity between human protein C and fibrinogen as well as between human protein C and Factor VIII is low. Additionally, plasma contains a variety of proteins, making it even more markedly different from activated protein C. Furthermore, activated protein C has distinct post-translational modifications – including addition of oligosaccharides onto four *Asn*-glycosylation sites – and a particularized glycosylation pattern – for example, contains *N*-acetylgalactosamine (GalNAc) in its Asn-linked oligosaccharides – that further distinguish it from the proteins in the '645 patent (Yan *et al.*, *Glycobiology*, 3: 597-608 (1993)).

7. Therefore, I conclude:

(a) The '650 patent only refers to lyophilization – not cryogranulation – of the protein C or activated protein C of that patent's invention. Additionally, the '645 patent uses lyophilization for fibrinogen. Since lyophilization and cryogranulation are two distinct processing techniques as detailed above, neither the '650 patent nor the '645 patent describes

or suggests the activated protein C cryogranules or the cryogranulation of activated protein C of the present invention; and

(b) The '645 patent's use of cryoprecipitation on plasma to prepare fibrinogen that is mostly free of Factor VIII does not suggest or predict the viability of cryogranulating a different protein, such as activated protein C, as detailed above.

8. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Jeffrey Clayton Baker

JEFFREY CLAYTON BAKER

May 18, 2004

Date

UNITED STATES OF AMERICA

STATE OF INDIANA)
) SS:
COUNTY OF MARION)

Before me, a Notary Public for Marion County, State of Indiana, personally appeared JEFFREY CLAYTON BAKER and acknowledged the execution of the foregoing instrument this 18th day of MAY, 2004.

Audrey J. Tuschum

Notary Public

My commission expires March 26, 2008

CURRICULUM VITAE

JEFFREY CLAYTON BAKER

Present Address

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Indianapolis, Indiana 46205
USA

Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285 USA

Education

North Texas State University, Denton, Texas
Received Ph.D. in Biochemistry, 1984

Northwestern University, Evanston, Illinois
Received B.A. in Biochemistry and Molecular Biology, 1980

Denton High School, Denton Texas
Graduated with Honors, 1976

Honors and Awards

2000 Lilly Research Laboratories President's Recognition Award (Development and transfer to manufacturing of the Xigris process)

1995 Lilly Research Laboratories President's Recognition Award (Crystallization of Insulin-lispro)

1982-1984 Samuel Roberts Noble Predoctoral Fellowship

1981 Robert A. Welch Predoctoral Fellowship

1980 Latham Scholar Award (outstanding undergraduate research)

Professional Affiliations

Advisor, Biotechnology Training Program, Indiana University School of Medicine

American Chemical Society

Institute of Validation Technology

International Society of Pharmaceutical Engineers

PDA (International Association for Pharmaceutical Science and Technology)
Chair, 2004 Biopharm Program Committee

Technical Leadership Experience

April 2004- present: Research Advisor

March 2002- April 2004: Senior Research Scientist-Validation, Manufacturing Science and Technology, Eli Lilly & Company. Responsible for the technical development and assessment of validation and qualification standards for global manufacturing. Member, Corporate Biotechnology Strategy Review Board.

August 2001- March 2002: Quality Advisor-Validation, Global Quality Policy and Technical Support, Eli Lilly and Company. One of 8 senior contributors identified to form a global technical policy and review group reporting through the Vice President for Quality at Lilly. Specific focus areas were bioprocess control and validation strategies and procedures. This group was dissolved as a result of corporate reorganization.

January 1997-August 2001: Senior Research Scientist, Bioprocess Commercialization, Eli Lilly and Company. Group Leader for development and registration of Xigris™ (activated protein C), a complex glycoprotein produced in cultured human cells. This role included supervision of laboratory and pilot plant activities, support of regulatory and clinical trial documentation, and coordination of tech transfer and launch activities with a contract manufacturer. Group representative to U.S. and European regulatory agencies. Lead development scientist for US and EU plant pre-approval inspections. Process Core Team Leader for post-registration process development strategies.

January 1993-1997: Research Scientist, Biochemical and Biosynthetic Isolation and Purification Development, Eli Lilly and Company. Responsibilities extended to technology transfer and CMC coordination within the insulin analog program as well as development of processes for purification of peptide anti-infectives.

November 1988-January 1993: Senior Biochemist, Biochemical and Biosynthetic Isolation and Purification Development, Eli Lilly and Company. Responsible for purification process development of insulin and insulin analogs at laboratory and pilot scales. Lead scientist for purification process development, tech transfer, and global product registration for Humalog™ (insulin-lispro).

October 1984-November 1988: Research Associate, Laboratory of Dr. Bruce N. Ames, Department of Biochemistry, University of California, Berkeley, CA. "Dinucleotide Metabolism, Oxidative Stress, and DNA Repair in Bacteria and Cultured Mammalian Cells"

June 1980-October 1984: Graduate Student in the Laboratory of Dr. Myron K. Jacobson, Department of Biochemistry, North Texas State University, Denton, TX. "Metabolism of Diadenosine Tetraphosphate in Cultured Mammalian Cells".

June 1979-June 1980: Research Assistant for Dr. J. W. Kauffman, Dept. of Biomedical Engineering, Northwestern University, Evanston, IL, and Dr. J. G. Garvin, Dept. of Biochemistry, Northwestern University Medical School, Chicago, IL. "Study of Membranes by Differential Scanning Calorimetry and Fourier Transform Infrared Spectroscopy".

Teaching Experience

2004 (spring): Visiting Professor, Graduate Program in Product Development, McCormick School of Engineering and Applied Science, Northwestern University, Evanston IL

2002: Guest Lecturer, Graduate Program in Bioseparations, Rose-Hulman Technological Insititute, Terre Haute, Indiana

2000: Co-developer of and lecturer in the "Biotech University" program at Eli Lilly & Company, Indianapolis IN, a comprehensive education and training program on Bioprocess development, manufacturing, and regulatory issues for management and staff.

1990-present: In-house Lecturer for annual classes on Protein Chemistry and BioSeparations, Eli Lilly and Company, Indianapolis IN.

1996-97: Guest Lecturer, Process Engineering of Renewable Resources, Graduate Engineering Program, Purdue University, West Lafayette IN

1990: Guest lecturer, Graduate Biochemistry at Indiana University/Purdue University at Indianapolis, Indianapolis IN

1987: Instructor, "Models in Biochemistry and Molecular Biology", Department of Biochemistry, University of California, Berkeley, CA.

1986: Instructor, "Transforming Growth Factors and Phenotypic Transformation", Department of Biochemistry, University of California, Berkeley, CA.

1980-81: Teaching Assistant, "Biochemistry Laboratory for Biochemistry Majors" and "Biochemistry Laboratory for Non-Majors", North Texas State University, Denton, TX.

1980: Teaching Assistant "Biochemical Research Techniques", Northwestern University, Evanston, IL.

Publications and Patents

Baker, J.C. and Jacobson, M. K. (1984) Determination of Diadenosine-5', 5'"- P1, P4-Tetraphosphate Levels In Cultured Mammalian Cells. *Analyt. Biochem.* **141**, 451-460. (A preliminary report of this work appeared in *Fed. Proc.* **42**:1144 (1983).)

Baker, J.C. and Jacobson, M. K. (1984) Analytical Approaches to the Study of Ap4A Metabolism in Intact Cells. *Hoppe-Seyler's Z. Physiol. Chem.* **365**, 602-603.

Baker, J.C. and Jacobson, M. K. (1986) Alterations of Adenyl Nucleotide Metabolism by Environmental Stress. *Proc. Nat. Acad. Sci. USA* **83**, 2350-2352. (A preliminary report of this work appeared in *Fed. Proc.* **43**:1728 (1984).

Publications and Patents (continued)

Yamamoto Y., Brodsky, M.H., Baker, J.C., and Ames, B.N. (1987) Detection and Characterization of Lipid Hydroperoxides at Picomole Levels by High Performance Liquid Chromatography. *Analyt. Biochem.* **160**, 7-13.

Baker, J.C., Smale S., Tjian R. and Ames, B.N. (1987) Inhibition of SV40 DNA Replication In Vitro by Poly(ADP-ribosyl)ated Diadenosine Tetraphosphate. *J. Biol. Chem.* **262**, 14855-14858.

Baker, J. C. and Ames, B. N. (1988) Alterations of 5'-Adenyl Nucleotides Following DNA Damage in Normal Human Fibroblasts and Fibroblasts Derived From Patients with Xeroderma Pigmentosum. *Mutation Res.* **208**, 87-93

Baker, J. C. and Ames, B. N. (1988) Alterations of NAD and 5'-Adenyl Nucleotides in Chediak-Higashi Syndrome Fibroblasts. *J. Inherit. Metab. Dis.* **11**, 221-228.

Kramer, G. F., Baker, J. C. and Ames, B. N. (1988) Near-UV Stress in Salmonella: 4-Thiouridine in tRNA, ppGpp, and ApppGpp as Components of an Adaptive Response. *J. Bacteriol.* **170**, 2344-2351.

Baker, J.C., Smale S., and Ames, B.N. "Inhibition of SV40 DNA Replication In Vitro by Poly(ADP-ribosyl)ated Diadenosine Tetraphosphate" in *ADP-Ribose Transfer Reactions: Mechanisms and Biological Significance*, M.K. Jacobson and E.L. Jacobson, eds. Sprenger-Verlag, 1989.

Long, H.B., Baker, J.C., Belagaje, R.M., DiMarchi, R.D., Frank, B.H., Green, L.K., Hoffmann, J.A., Muth, W.L., Pekar, A.H., Reams, S.G., Shaw, W.n., Shields, J. E., Slieker, L.J., Su, K. S.E., Sundall, K., and Chance, R. "Human Insulin Analogs With Rapid Onset and Short Duration of Action." in *Peptides: Synthesis Structure, and Function*, John A. Smith and Jean A. Rivier, eds., ESCOM, 1992
(A Poster of the same title was presented at the 12th Annual Peptide Symposium in Boston MA, 1991)

Baker, J. C. (1992) "The Other Corner of the Triangle: Throughput Issues in Process Chromatography" *Abstr. Am. Chem. Soc.* **203**, 103 (Abstract of a seminar given at the 1992 ACS Convention, San Francisco CA)

Ciszak, E., Smith, D.G., Baker, J. C., Beals, J.M., Carter, N. D. , and Frank, B.F. (1994) "The Structure of Lys-Pro Insulin" , abstract and poster for presentation at the 1994 Meeting of the American Crystallographic Association, Atlanta GA.

Frank, B.F., Baker, J. C., Beals, J.M., Carter, N. D. , Ciszak, E., Pekar, A.H. and Smith, D.G., (1994) "The Structure of Lys-Pro Insulin" , abstract and poster for presentation at the 15th International Diabetes Federation Congress, Kobe, Japan.

Publications and Patents (continued)

Ciszak, E., Beals, J.M., Frank, B.F., Baker, J. C., Carter, N. D. , and Smith, D.G., (1995) Role of C-Terminal B-Chain Residues in Insulin Assembly: The Structure of Hexameric LysB28, ProB29-Human Insulin. *Structure* **3**: 615-622

Baker, J.C., Carter, N.D., and Frank, B.H., Preparation of Stable Zinc Insulin Analog Crystals, U.S. Patent No. 5,504,188, issued April 2, 1996.

Baker, J.C., and Roberts B.M., Preparation of Stable Insulin Analog Crystals, U.S. Patent No. 5,597,893, issued January 28, 1997.

Baker, J.C., Hanquier J.M., and Shrader, W.R. Reducing Gelation of a Fatty Acid Acylated Protein, U.S. Patent No. 5,631,347, issued May 20, 1997.

Baker, J.C., Chen, V.J., Hanquier, J.M., Kriauciunas, A., Moser, Brian A. And Shuman, R.T., Selective Acylation of e-Amino Groups, U.S. Patent No. 5,646,242, issued July 8, 1997.

Baker, J.C. and Hanquier, J.M., Acylated Insulin Analogs, U.S. Patent Number 5,693,609, issued December 7, 1997

Baker, J.C., Moser, B.A., and Shrader, W.R. Preparation of an Acylated Protein Powder, U.S. Patent No. 5,700,904, issued December 23, 1997

Baker, J.C. and Hanquier, J.M., Acylated Insulin Analogs, U.S. Patent Number 5,922,675, issued July 13, 1999

Baker, J.C., Carlson, A., Huang, L, and Sheliga T, Methods for Processing Activated Protein C, US Patent Number 6,162,629, Issued December 19, 2000

Baker, J.C., Carlson, A., Huang, L, and Sheliga T, Activated Protein C Formulations, US Patent Number 6,436,397, Issued August 20, 2002

Not noted in detail are Eli Lilly and Company internal development reports and guidance documents, contributions to the preparation of global regulatory commitment documents (5 IND's, 2 CTX's, 1 NDA, 2 EU Registry Dossiers, 2 Canadian NDA's, 1 Japanese NDA, 1 BLA, and supporting amendments) or presentations on the development of biosynthetic processes to domestic and European regulatory authorities.

Invited Seminars

"Metabolism of 5'-5'''-Adenylyl Dinucleotides", Department of Biochemistry, Medical College of Wisconsin, Milwaukee WI, 1989, also presented to the Department of Biochemistry, University of Texas Medical School, Galveston, TX, 1989

"The Other Corner of the Triangle: Throughput Issues in Process Chromatography", American Chemical Society annual meeting, San Francisco CA, 1992.

"Bioseparations", Introductory remarks (as section leader) to the Gordon Conference on Separation and Purification, 1992.

"Strategies in Biosynthetic Process Development", Department of Chemical Engineering, Purdue University, Lafayette, IN, 1992, also presented to the College of Pharmacy, University of Kentucky, Lexington, KY, 1994

"Advances in Large Scale Biosynthetic Processing: A Comparison of the Insulin and Insulin-lispro Processes" , Plenary Lecture at PrepTech '95, 1995

"Control of Impurities in Large Scale Biosynthetic Processing: Mapping Mountains and Molehills" , BioWest Conferences, San Jose, CA, 1995

"Strategies in Biosynthetic Process Development", Department of Chemical Engineering, University of Kentucky, Lexington, KY, 1996

"What is Biosynthetic Process Development?" Department of Chemical Engineering, Purdue University, West Lafayette IN, 1996

"The Other Corner of the Triangle: Throughput Issues in Process Chromatography", The Purdue University Chromatographic Workshop, West Lafayette Indiana, 1996

"Unlocking the Process: Post-Registration Changes to Commercial Bioprocesses", American Chem. Society, Recovery of Biological Products VIII, Tucson, AZ 1996

"Modeling Up and Scaling Down: Linking Bioprocess Development in the Laboratory, Pilot Plant, and Manufacturing Setting", Biologics & Pharmaceutical Contract Process Development Symposium, 1997, Bethesda MD

"aPC Commercial Purification Process Development", a Lilly Research Laboratories Grand Rounds Presentation, 1997

"LysPro Purification Process Development: Mapping Mountains and Molehills", PhRMA Biological and Biotechnology Committee 1997 Spring Meeting, Philadelphia PA

Invited Seminars (continued)

"Large Scale Purification of Therapeutic Peptides: Preparation of Glandular Pork Insulin, Recombinant Human Insulin, and the Insulin Lis-Pro Analog" Purdue University Chromatography Workshop, 1997

"Inter-company Development Issues", a workshop developed and chaired for the American Chemical Society, Recovery of Biological Products IX, Whistler, BC 1999

"Distal Impact: Inclusion Body Isolation and Downstream Chemistries" American Chemical Society Annual Meeting, Symposium on Interacting Bioprocess Steps, San Francisco CA, 2000Center for Advanced Biochemical Engineering, University College, London, UK 2000

"First You Need a Map: Transferring Bioprocesses Between Sites and Organizations" International Symposium on Site Transfer, San Francisco CA, 2000

"Large Scale Purification of Therapeutic Peptides: Preparation of Glandular Pork Insulin, Recombinant Human Insulin, and the Insulin Lis-Pro Analog" 13th Colorado Biotechnology Symposium, Colorado State University, Ft Collins, CO, 2000

"Modeling Up and Scaling Down: Linking Laboratory, Pilot Scale, and Commercial Bioprocessing" , plenary session presentation, PDA/FDA Conference on Validation of Manufacturing Processes for Biologics, Washington, DC, 2000

"Weighing the Wait: Considerations in Dynamic Process Modeling", Symposium on Transitions from Bench to Clinic, Boston, MA, 2001

"First You Need a Map: Linking Laboratory, Pilot Scale, and Commercial Bioprocess Operations", University of Michigan Pharmaceutical Engineering Seminar Series, Ann Arbor MI, 2001

"Learning Points from the Xigris Pre-Approval Inspection" Bioprocess Research and Development Seminar Series, Eli Lilly and Co, 2002

"Gray Questions in Bioprocess Validation" A workshop developed with Dr. John Finkbohner, Deputy Director, FDA, GMP by the Sea, Cambridge MD 2003

"Post-registration Changes in Insulin Manufacturing: 80 years of Learning and Improvement" IBC Conference on Production and Economics of Biopharmaceuticals" San Diego CA, 2003